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Effects of Natural or Synthetic Microbial Adjuvants on Induction of Autoimmune Thyroiditis

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Natural and synthetic adjuvants of microbial origin were compared for their capacity to potentiate the induction of experimental autoimmune thyroiditis (EAT) with the autoantigen mouse thyroglobulin (MTg). Regardless of the immunomodulator used, severe thyroiditis was observed only in EAT-susceptible strains of the *k* haplotype and not in EAT-resistant strains of the *d* haplotype. Compared to phenol-extracted lipopolysaccharide, a potent adjuvant for enhancing EAT induction, phthalyl-substituted, detoxified lipopolysaccharide, even at doses 15- to 50-fold greater, led to only low anti-mouse thyroglobulin titers and mild thyroid infiltration. The synthetic adjuvant *N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) and three of its analogs, *N*-acetylmuramyl-L-alanyl-D-isoglutamine-L-alanyl-D-glycerol mycolate (MDP-L-Ala-Glyc-Myc), *N*-acetylmuramyl-L-alanyl-D-glutamyl-(decyl)methyl ester [MDP(decyl)methyl], and *N*-acetylmuramyl-L-alanyl-D-glutamine-α*n*-butyl ester [MDP-(Gln)-O*n*Bu], designated murabutide, were tested in incomplete Freund adjuvant or in saline. In incomplete Freund adjuvant, MDP-L-Ala-Glyc-Myc was inefficient in inducing EAT, murabutide induced very mild involvement, and MDP and, more so, MDP(decyl)methyl were active but to a lesser degree than CFA. When saline was used, low levels of thyroid infiltration were observed in a few of the MDP-treated animals in only one experiment, whereas no lesions were observed when murabutide was used.

Many parameters are used to assess the suitability of an adjuvant for human prophylaxis and therapy. A very important determinant among them is whether the adjuvant activates the response to autologous antigens. Experimental autoimmune thyroiditis (EAT) in mice provides a particularly suitable model for gauging the role of adjuvants in potentiating autoimmunity. Its linkage to certain haplotypes of the mouse major histocompatibility complex, the *H-2* complex (32), has provided clues for later studies of the association of a number of human autoimmune diseases with *HLA-D/DR* (15). As in human chronic lymphocytic thyroiditis (Hashimoto's disease), EAT is characterized by both antibody production to the inducing autoantigen, mouse thyroglobulin (MTg), and infiltration of the thyroid by mononuclear cells, including lymphocytes and macrophages. The disease is induced only in the presence of T cells from high-responder, susceptible strains (33). Recently, the immune response gene (*Ir-Tg*) was mapped to the *I-A* subregion (2), but the *D* end (17) and non-*H-2* genes (3) may modify the severity of thyroiditis or antibody levels, respectively, or both. In all probability, the polygenic influence resembles that in humans.

In susceptible strains, both complete Freund adjuvant (CFA) and bacterial lipopolysaccharide (LPS) serve as potent adjuvants for MTg, resulting in thyroid lesions that are absent in control animals given comparable doses of MTg alone (14). Moreover, high-responder T cells are also required when LPS is used as an adjuvant. Polyadenylic acid-polyuridylic acid complex [poly(A-U)] is less potent as a T-cell adjuvant for EAT, primarily aiding in the production of antibodies in high-responder mice (13).

In this report, we compared the adjuvant LPS with a derivative of LPS which was obtained by phthalylation (sodium phthalyl LPS, SPLPS). SPLPS has lost its toxicity, as shown in animals (5) and humans (10), but retains its adjuvant activity (5). We also compared CFA with an emulsion of *N*-acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide, MDP), the minimal synthetic structure which can replace mycobacteria in CFA (11, 19). MDP is adjuvant active in several models (1, 21) and is also capable of enhancing nonspecific immunity (6). In recent years, the synthesis of hundreds of MDP derivatives has permitted the study of the relationships between structure and biological activities. In this report, we included a lipophilic derivative, *N*-acetylmuramyl-L-alanyl-D-isoglutamine-L-alanyl-D-glycerol mycolate (MDP-L-Ala-Glyc-Myc), a highly hydrophilic analog, *N*-acetylmuramyl-L-alanyl-D-glutamyl-(decyl)methyl ester [MDP(decyl)methyl], and a derivative called murabutide, *N*-acetylmuramyl-L-alanyl-D-glutamine-α*n*-butyl ester [MDP-(Gln)-O*n*Bu]. Murabutide is as active as MDP as an adjuvant but is devoid of any detectable side effects, including pyrogenicity (7), and is undergoing clinical trials.

MATERIALS AND METHODS

Animals. Female high-responder (*H-2*^{k}) and low-responder (*H-2*^{d}) mice were used at 8 to 10 weeks of age. For comparison, three strains of the *k* haplotype and two strains of the *d* haplotype were studied. The three *k* strains were B10.BR and CBA/J, from the Jackson Laboratory, Bar Harbor, Maine, and C3H/HeN, kindly supplied by Ken Morrison (formerly of the Department of Immunology and Microbiology, Wayne State University). The two *d* strains were B10.D2, from the Jackson Laboratory, and BALB/c, maintained at Wayne State University. In some experiments carried out at the Pasteur Institute, the CBA mice were purchased from Iffa-Credo, L'Arbresle, France.

Thyroglobulin. MTg was prepared from frozen mouse thyroids (Pel-Freez Biologicals, Inc., Rogers, Ark.) as de-

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TABLE 1. Effect of different adjuvants on the kinetics of the MTg antibody response and thyroid infiltration in high- and low-responder mice

Strain	Treatment[a]	Mean $\log_2$ titer ± SE on day:				Thyroiditis[b]	
		7	14	21	28	No. positive/ total no. tested	Pathology index
B10.BR (*H-2*^{k})	MTg	<1.0	<1.0	<1.0	<1.0	0/3	0.0
	MTg + poly(A-U)	1.0[c] ± 0.4	7.3 ± 0.2	6.5 ± 0.5	5.8 ± 0.4	1/5	0.2 ± 0.1
	MTg + LPS	5.8[c] ± 0.5	10.0 ± 0.6	9.6 ± 0.2	9.8 ± 0.5	6/6	1.5 ± 0.2
	MTg in CFA	<1.0	8.0 ± 0.7	8.8 ± 0.5	11.0 ± 0.7	2/3	1.0 ± 0.6
B10.D2 (*H-2*^{d})	MTg	<1.0	<1.0	<1.0	<1.0	0/4	0.0
	MTg + poly(A-U)	<1.0	<1.0	<1.0	<1.0	0/5	0.0
	MTg + LPS	<1.0	9.4 ± 0.4	8.0 ± 0.3	8.2 ± 0.7	0/6	0.0
	MTg in CFA	<1.0	<1.0	10.3 ± 0.7	11.2 ± 0.8	0/5	0.0

[a] MTg (20 μg) was given i.v. on days 0 and 7; 300 μg of poly(A-U) and 20 μg of LPS were injected i.v. 3 h later; and MTg in CFA was injected s.c. on days 0 and 7.
[b] Animals were killed for histologic examination on day 28.
[c] Sensitive to 2-mercaptoethanol.

scribed previously (17). Briefly, the thyroids were freed from the tracheae, trimmed, and gently disrupted in borate-buffered saline (pH 8.2) with a glass homogenizer. The extract was centrifuged at 100,000 × *g* for 60 min and column fractionated (Sephadex G-200; 2.5 × 90 cm). The concentration of MTg was determined spectrophotometrically at 280 nm, and its purity was verified by immunoelectrophoresis at a concentration of 10 to 15 mg/ml against our rabbit antiserum to crude thyroid extract and against rabbit antimouse serum obtained from Miles Laboratories, Inc., Elkhart, Ind. MTg was stored in aliquots at concentrations of 1 to 2 mg/ml at −20°C.

Adjuvants. *Salmonella enteriditis* LPS was prepared by precipitation with trichloroacetic acid and was kindly supplied by C. D. Jeffries (Department of Immunology and Microbiology, Wayne State University). *S. enteriditis* LPS W, obtained by phenol extraction (Difco Laboratories, Detroit, Mich.) was used to prepare SPLPS by a slight modification (5) of the method of Schenck et al. (29). The final product was 10,000-fold less toxic than native LPS, as shown by testing for toxicity in adrenalectomized mice and for pyrogenicity in rabbits, but remained adjuvant active (5). MDP was MDP-Girpi. MDP-L-Ala-Glyc-Myc and MDP(decyl)methyl were prepared by previously described methods (11, 23). Murabutide, MDP-(Gln)-O*n*Bu, was synthesized as described by Lefrancier et al. (22). All preparations were dissolved in nonpyrogenic phosphate-buffered saline (pH 7.2). SPLPS was dispersed with a Vortex mixer to aid solubilization.

CFA was specially prepared and emulsified with MTg at a ratio of 1:2 as described previously (14). Synthetic glycopeptides were used similarly in place of mycobacteria. Incomplete Freund adjuvant (IFA) contained no mycobacteria or glycopeptides. In later experiments, IFA was obtained from Difco Laboratories and used at a 1:1 ratio with MTg, with similar results. Polyadenylic acid and polyuridylic acid (Miles Laboratories) were dissolved in nonpyrogenic saline separately; poly(A-U) was polymerized by mixing before use (16).

Induction of autoimmune thyroiditis. MTg and all adjuvants were diluted in nonpyrogenic saline. EAT was induced by injecting 20 to 150 μg of MTg in CFA subcutaneously (s.c.) or 20 μg of MTg intravenously (i.v.) followed by 20 μg of LPS i.v. 3 h later on days 0 and 7 (14, 17). Like LPS, SPLPS was injected i.v. in doses of 300 μg or 1 mg, and poly(A-U) was injected in doses of 300 μg (13). MDP analogs were injected either i.v. or s.c. in IFA.

Assay procedures. Weekly serum samples were obtained from the tail artery beginning on day 7, just before the second immunizing dose. Antibody titers to MTg were determined with or without 2-mercaptoethanol by passive hemagglutination as described previously (12). The animals were killed on the last day of antibody determination, and the entire thyroid was removed and sectioned for histologic examination. The degree of mononuclear infiltration, expressed as the average pathology index of the group, was graded as follows: 0, no infiltration; 0.5, small focal areas of infiltration; 1, infiltration with follicular destruction; 2, up to 40% infiltration; and 3, 40 to 80% infiltration (28). The numbers of animals that had definite focal areas of infiltration in the thyroid were also recorded.

RESULTS

Effect of different adjuvants on the genetic control of autoimmunity to MTg. Earlier studies with three different adjuvants, CFA, LPS, and poly(A-U), indicated that genetic control of the *H-2*-linked, T-cell-mediated response to MTg is independent of the adjuvant used (13, 14, 32). However, the vigor of the autoimmune response, at a constant antigen dose, appears to be governed by the potency of the adjuvant. To verify and extend these observations, we immunized congenic mice differing only at the *H-2* complex on days 0 and 7 with 20 μg of MTg alone or with poly(A-U), LPS, or CFA as the adjuvant. The kinetics of the antibody response and the severity of thyroid infiltration in the high-responder B10.BR (*H-2*^{k}) and low-responder B10.D2 (*H-2*^{d}) strains were monitored. The genetically determined difference held fast according to the haplotype (Table 1). As predicted by the *H-2* linkage (32), severe thyroiditis was present only in high-responder B10.BR mice. In agreement with earlier findings, both LPS and CFA were effective adjuvants in aiding in the induction of thyroid lesions in B10.BR mice, whereas poly(A-U) was primarily effective in enhancing the development of antibodies to MTg, albeit at lower levels. Antibody production occurred earlier in high-responder mice; after only one injection of MTg with poly(A-U) or LPS, low titers of mercaptoethanol-sensitive (usually immunoglobulin M) antibodies were observed on day 7. In all

TABLE 2. Effect of detoxified LPS on the kinetics of the MTg antibody response and thyroid infiltration in high-responder mice[a]

Treatment[b]	Mean log_2 titer ± SE on day:				Thyroiditis	
	7	14	21	28	No. positive/ total no. tested	Pathology index
MTg (20 μg) + SLPLS (300 μg)	<1.0	<1.0	1.2 ± 0.3	0.8 ± 0.5	3/6	0.7 ± 0.5
MTg (20 μg) + SPLPS (1 mg)	<1.0	<1.0	2.2 ± 0.5	3.2 ± 0.1	4/6	0.5 ± 0.2
MTg (20 μg) + phenol-extracted LPS (20 μg)	3.4[c] ± 0.5	8.0 ± 0.5	9.0 ± 0.3	11.0 ± 0.6	7/7	2.1 ± 0.1

[a] C3H/HeN (*H-2*^{k}) mice, killed on day 28 for histologic examination.
[b] MTg was given i.v. on days 0 and 7; the adjuvant was given i.v. 3 h later.
[c] Sensitive to 2-mercaptoethanol.

groups displaying antibodies, switching to the immunoglobulin G class was observed. Although immunoglobulin G antibodies to MTg were also present in low-responder B10.D2 mice treated with LPS or CFA, they did not, as a rule, correlate with lesions (13, 14).

Effect of detoxified LPS (SPLPS) on autoimmunity to MTg. Since B10.BR mice were susceptible to EAT regardless of the adjuvant used, additional strains bearing *H-2*^{k} were used to test the other adjuvants. The LPS-responsive strain C3H/HeN was used to determine if, after phthalyl substitution, adjuvant activity was retained for EAT. In contrast to LPS, SPLPS has been shown to be nonpyrogenic for adrenalectomized mice (5) and for humans (10) but to retain its adjuvant activity in mice (5). Two groups of mice were immunized on days 0 and 7 with 20 μg of MTg, followed 3 h later by 300 μg or 1 mg of SPLPS. LPS extracted with phenol (the parent compound) was included as a control. In contrast to LPS treatment, which resulted in 100% of the animals having marked thyroid infiltration, treatment with SPLPS at either dose led to very low anti-MTg titers (Table 2). Little difference in the percentage of animals with thyroid lesions was observed between the two SPLPS-treated groups, and the thyroiditis in either group was not severe (average indices of 0.5 to 0.7). Similar results were observed with CBA mice, also of the *k* haplotype (data not shown). Thus, SPLPS, although nontoxic, served as a weak adjuvant for EAT.

Effect of MDP and its analogs on autoimmunity to MTg. The capacity of MDP both in the aqueous form and in IFA to aid in the induction of EAT was examined in the first series of experiments. MDP, previously shown to be an effective adjuvant for many antigens (1, 21), was administered s.c. at 100 μg. The experiments were carried out in both high-responder CBA and low-responder BALB/c mice and terminated on day 14 or 28. Compared to CFA, MDP in IFA was less effective as an adjuvant in inducing thyroiditis (Table 3). On day 14, only 42% (5 of 12) of the CBA mice in the group treated with MDP in IFA, compared with 100% (6 of 6) of the CFA-treated mice, had lesions which were less severe (average index, 0.4 versus 1.9). In mice killed on day 28, however, 83% (five of six) had lesions with an average index of 1.0, indicating that a longer period of stimulation could lead to a higher incidence of thyroiditis. It should be noted that MTg in IFA without mycobacteria has never produced

TABLE 3. Effect of MDP alone or with IFA or of CFA as the adjuvant on the kinetics of the MTg antibody response and thyroid infiltration in high- and low-responder mice

Strain	Treatment[a]	Mean log_2 titer ± SE on day:				Thyroiditis[b]	
		7	14	21	28	No. positive/ total no. tested	Pathology index
CBA (*H-2*^{k})	MTg (20 μg) in CFA	1.2[c] ± 0.3	5.2 ± 0.8			6/6	1.9 ± 0.3
	MTg (20 μg) + MDP (100 μg) in IFA	1.4[c] ± 0.2	4.7 ± 0.6			5/12	0.4 ± 0.1
	MTg (20 μg) + MDP (100 μg) in IFA	<1.0	9.3 ± 0.5	10.5 ± 0.3	13.2 ± 0.3	5/6	1.0 ± 0.3
	MTg (20 μg) + MDP (100 μg)	1.7[c] ± 0.3	3.7 ± 0.1			4/12	0.2 ± 0.1
	MTg (20 μg) + MDP (100 μg)	1.2[c] ± 0.2	5.2 ± 0.2	4.0 ± 0.5	4.8 ± 0.6	4/5	0.7 ± 0.3
BALB/c (*H-2*^{d})	MTg (20 μg) in CFA	<1.0	2.2 ± 0.9			1/6	0.2 ± 0.1
	MTg (20 μg) + MDP (100 μg) in IFA	<1.0	<1.0			0/6	0.0
	MTg (20 μg) + MDP (100 μg) in IFA	<1.0	5.0 ± 0.2	4.3 ± 0.2	6.6 ± 0.7	1/7	0.1 ± 0.1
	MTg (20 μg) + MDP (100 μg)	<1.0	1.4[c] ± 0.0			0/5	0.0
	MTg (20 μg) + MDP (100 μg)	<1.0	2.8 ± 0.2	1.0 ± 0.7	<1.0	0/6	0.0

[a] MTg was given i.v. and the adjuvant was given s.c. on days 0 and 7.
[b] Animals were killed for histologic examination on the day of the last antibody determination.
[c] Sensitive to 2-mercaptoethanol.

TABLE 4. Effect of MDP analogs alone or with IFA as the adjuvant on the kinetics of the MTg antibody response and thyroid infiltration in high-responder mice[a]

Treatment			Mean log_2 titer ± SE on day:				Thyroiditis[b]	
MTg (μg)	MDP[c] or analog[c]	IFA	7	14	21	28	No. positive/ total no. tested	Pathology index
20	MDP-L-Ala-Glyc-Myc (100 μg)	+	<1.0	3.6 ± 0.6			0/6	0.0
	MDP(decyl)methyl (100 μg)	+	<1.0	4.5 ± 0.5			6/6	1.3 ± 0.2
50	MDP (100 μg)	−	<1.0	4.4 ± 2.1	8.6 ± 1.7	10.4 ± 1.2	0/5	0.0
	MDP-L-Ala-Glyc-Myc (100 μg)	−	<1.0	2.0 ± 0.8	6.0 ± 1.7	8.0 ± 1.1	1/5	0.2 ± 0.2
	MDP(decyl)methyl (100 μg)	−	<1.0	<1.0	3.0 ± 0.8	6.2 ± 0.3	0/6	0.0
	MDP-(Gln)-O*n*Bu (100 μg)	−	<1.0	2.7 ± 1.7	8.8 ± 2.1	9.8 ± 1.4	0/6	0.0
150	MDP-L-Ala-Glyc-Myc (100 μg)	+		6.0 ± 3.3	14.0 ± 0.9		0/5	0.0
	MDP(decyl)methyl (100 μg)	+		16.7 ± 1.1	18.2 ± 1.5		4/6	0.9 ± 0.4
	MDP-(Gln)-O*n*Bu (100 μg)	+		16.0 ± 1.1	17.0 ± 1.6		2/4	0.4 ± 0.2

[a] Control CBA (*H-2*^{k}) mice given 20 μg of MTg in CFA had an antibody titer of 3.8 ± 0.4 and a pathology index of 1.0 ± 0.5 (five positive/six tested), on day 14; those given 150 μg of MTg in CFA had an antibody titer of 20.0 ± 3.1 and a pathology index of 2.7 ± 0.7 (three positive/three tested) on day 21.
[b] Animals were killed for histologic examination on the day of the last antibody determination.
[c] The aqueous form was given i.v., and the emulsion was given s.c.

any lesions in high-responder mice despite high antibody levels (13). Aqueous MDP injected in conjunction with soluble MTg resulted in low antibody titers and a low degree of infiltration in four of five animals (day 28). In contrast, low-responder mice given MTg in either CFA or MDP in IFA showed no or a very low incidence of infiltration; those given MTg in aqueous MDP transiently showed low antibody titers and no infiltration.

In the next set of experiments, three MDP analogs were tested with or without IFA, again with the rationale that they may act as an adjuvant for EAT when in soluble form or may require the assistance of IFA when in the form of an emulsion. Aqueous MDP was given i.v., an effective route for LPS and poly(A-U) (13, 14; see also Table 1). Higher doses of MTg were tested in some instances to compare the analogs to each other.

Table 4 is a composite of selective comparisons to illustrate three points. (i) All three MDP analogs, like MDP, could serve as adjuvants in the aqueous form by augmenting the production of antibodies to MTg. (ii) MDP(decyl)methyl in IFA was the most effective adjuvant in evoking significant levels of infiltration, whether the mice were examined on day 14 (six of six) or day 21 (four of six); nevertheless, it was less active than CFA (see Table 4). (iii) MDP-L-Ala-Glyc-Myc was the least efficacious adjuvant, whereas MDP-(Gln)-O*n*Bu appeared to be weakly active. None of the three analogs given with antigen in saline resulted in thyroid infiltration.

DISCUSSION

In the studies described above, we examined the potential of an immunomodulator to promote the autoantigenicity of MTg. As shown earlier with some of the adjuvants tested (13, 14, 27, 32), genetic susceptibility to EAT is consistently major histocompatibility complex linked. Regardless of the adjuvants used, severe thyroid infiltration was observed only in susceptible strains of the *k* haplotype and not in resistant strains of the *d* haplotype (Tables 1 and 3). This correlation enables an evaluation to be made of the potency of several immunomodulators and of the effect of chemical modification on their adjuvanticity. For induction of EAT, unmodified LPS again proved to be as efficacious as CFA (14) specially concocted to enhance thyroid lesions (32). Aqueous poly(A-U) and MDP were much less efficacious in promoting cellular infiltration. Whereas CFA is not a potential candidate for human use because of its well known side effects, poly(A-U) has been shown to enhance tumor regression in human trials (20) as well as to interfere with the activation of suppressor cells in murine EAT (18). The high toxicity of LPS makes it unsuitable for human use; however, it can be detoxified by chemical means (5, 10, 29). MDP has been shown to be pyrogenic at high doses, but several analogs and particularly murabutide have no detectable side effects (9, 22).

LPS has been shown to require T cells for its adjuvant activity for MTg (14). Unlike CFA, it can be given separately from soluble, unaltered MTg, thereby enabling autoreactive T cells to respond to native antigen. Thus, LPS from several sources, such as the gastrointestinal tract or an exogenous infection, could, on various occasions, serve as a natural adjuvant for the normally circulating thyroglobulin in a genetically susceptible host. It was therefore of interest to evaluate detoxified LPS as an adjuvant for EAT induction. Phthalyl substitution of LPS results in the loss of its toxicity for adrenalectomized mice but in the retention of its capacity to augment antibody production (5) and CFU [C. Bernard, F. Uttwiller, L. Chedid, C. Dresch, G. Hauptmann, A. Boilletot, C. Giron, J. P. Bergerat, J. M. Lang, and F. Oberling, Exp. Hematol. 9(Suppl.):20, 1981]. It is nonpyrogenic in humans even at a dose of 10 μg/kg, whereas LPS at a dose of 0.1 to 0.5 ng/kg elicits symptoms of endotoxicity (10). SPLPS also enhances the number of circulating lymphocytes in normal subjects and patients (10). In EAT-susceptible mice, SPLPS is apparently a weak adjuvant, despite a dose 15-fold higher than that of LPS (Table 2). A further increase of threefold to 1 mg of SPLPS did little to augment its capacity to raise levels of antibodies to MTg or the severity of thyroiditis. The combined observations in humans and mice point to the potential of SPLPS as an immunomodulator.

The synthetic adjuvant MDP in the aqueous form was weakly efficacious for EAT induction (Tables 3 and 4). The incorporation of MDP and MTg into IFA resulted in higher levels of antibodies to MTg but was less effective than mycobacteria in IFA in increasing thyroid infiltration. MDP in IFA with the appropriate antigen has served as an

adjuvant for autoimmune orchitis (30) and experimental allergic encephalomyelitis (24) in guinea pigs. In addition to MDP, we tested three other analogs with various biological activities. Like MDP, all three in the aqueous form promoted the formation of MTg autoantibodies but had a negligible effect on thyroid lesions. When they were incorporated into IFA and used with a high dose of antigen, higher MTg titers were observed with all three, but only the hydrophilic analog MDP(decyl)methyl in IFA was a potent stimulator of EAT. The lipophilic derivative MDP-L-Ala-Glyc-Myc was shown to be an ineffective analog, although it can enhance the delayed-hypersensitivity response to protein antigens and can stimulate nonspecific resistance (25). Murabutide was intermediate in potency; it has known adjuvant activity and enhances nonspecific resistance without concomitant pyrogenicity (6). It is well tolerated in humans (F. Oberling, C. Bernard, L. Chedid, J. Choay, C. Giron, and J. M. Lang, International Symposium on Immunomodulation by Microbial Products and Related Synthetic Compounds **IX-6**:41, 1981) and is undergoing clinical trials. Its intermediate effect on EAT induction in the presence of IFA and its augmentation of only autoantibody formation in the aqueous form make it an immunostimulator with considerable potential.

Questions may be raised concerning the safety of an adjuvant that enhances MTg autoantibody production. This concern may be greatly reduced if we take into consideration two important features of autoimmune thyroiditis. (i) In both humans (4) and mice (13, 14, 26), the mere presence of thyroid autoantibodies is not indicative of disease, nor is the level coincident with severity. The lack of correlation is again borne out by the data for MDP and its analogs in this study (Tables 3 and 4). (ii) Significant levels of MTg autoantibodies are produced in susceptible strains only when exogenous MTg is also given with an aqueous adjuvant (LPS and IFA are excluded here since they are unlikely to be used in humans). Neither CFA (8) or LPS (13) given alone nor a chronic graft-versus-host reaction (31) is sufficient to activate autoreactive T and B cells to respond to normal levels of circulatory MTg. Recent studies show these levels to be on the order of 50 to 100 ng/ml (M. Lewis, A. A. Giraldo, and Y. M. Kong, Fed. Proc. **43**:1995, 1984). Also, MDP or murabutide, given biweekly to EAT-susceptible mice over a period of 5 months, does not lead to the spontaneous appearance of MTg autoantibodies or thyroid lesions (unpublished observations). It has recently been reported that when circulatory MTg levels are raised by injecting thyroid-regulating hormones to levels two- to fourfold above normal, specific regulatory mechanisms suppressing EAT induction are activated (18; M. Lewis, A. A. Giraldo, and Y. M. Kong, Fed. Proc. **43**:1995, 1984). Interestingly, when LPS is also given with hormones, MTg levels are further increased, resulting in a more pronounced suppression (M. Lewis, A. A. Giraldo, and Y. M. Kong, Immunobiology **167**:44–45, 1984). Thus, nature appears to provide safeguards against periodic surges in the levels of autoantigen or adjuvant arising from microbial infections. Such homeostatic regulatory mechanisms would reduce the risk in the use of immunomodulators.

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